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Effect of Chrysotile Asbestos and Silica on the Microsomal Metabolism of Benzo(a)pyrene

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The effects of chrysotile, water-leached chrysotile, and silica on microsomal metabolism of benzo(a)pyrene *in vitro* were studied. Examination of benzo(a)pyrene metabolites generated by 3-methylcholanthrene-pretreated rat liver microsomes, in the presence of chrysotile fibers, revealed a reduction in the overall metabolism of the hydrocarbon. Thus, chrysotile appeared to modify the activities of aryl hydrocarbon hydroxylase and epoxide hydrolase. Leaching chrysotile in deionized water for 24 hr elicited a similar response. Silica, in contrast to chrysotile, did not decrease the microsomal metabolism of benzo(a)pyrene. Chrysotile, as well as water-leached chrysotile, considerably diminished the microsomal production of water-soluble benzo(a)pyrene metabolites. Precoating the fibers with heparin or bovine serum albumin partially abolished this inhibition. The liver microsomal metabolism of benzo(a)pyrene in rats subjected to intraperitoneal administration of chrysotile was 58% of that in untreated rats.

Introduction

Exposure to complex chemical mixtures is usually considered to induce neoplastic transformation of the respiratory tract epithelium, termed as bronchogenic carcinoma (*1*). The best illustrated example is cigarette smoke which, on the basis of several epidemiological studies, is regarded as the main etiological agent in the induction of bronchogenic carcinoma (*2*). However, the genesis of lung cancer seems to be associated with the combined exposure of cigarette smoke and other factors such as particulates (*1*). These agents could conceivably have a promoting role in respiratory tract carcinogenesis. For instance, it is well known that exposure to asbestos fibers could greatly augment the risk of bronchogenic carcinoma posed by cigarette smoke (*3*). This action would explain the synergistic effect of asbestos inhalation and cigarette smoking, as exemplified by an increased incidence of pulmonary carcinoma in smoking asbestos workers (*4*). Experimental studies also show that simultaneous exposure to polycyclic aromatic hydrocarbons, known to be present in cigarette smoke, and asbestos results in synergistic or cocarcinogenic effects (*5, 6*). Even though asbestos appears to possess only weak carcinogenic activity, it can strikingly aggravate the action of carcinogenic hydrocarbons introduced into the respiratory tract (*7, 8*). The cellular and molecular bases of the interaction between polycyclic aromatic hydrocarbons and asbestos remain to be understood. It is conceivable that the fiber may adsorb the hydrocarbons onto its surface, thus facilitating their uptake into the tissues. Alternatively, asbestos fibers may directly modify carcinogen-metabolizing aryl hydrocarbon hydroxylase systems, thus, possibly leading to an increased production of ultimate carcinogenic forms of polycyclic aromatic hydrocarbons. Our earlier reports indicated that asbestos inhibited benzo(a)pyrene hydroxylase activity *in vitro*. If the fibers could inhibit the metabolism of benzo(a)pyrene *in vivo*, it would prolong the residence time of the carcinogen in the tissue. In the current study we have examined the effects of pretreatment of rat liver microsomes with chrysotile, water-leached, chrysotile, and silica on the overall metabolism of benzo(a)pyrene. We have also studied the effect of chrysotile asbestos administration to rats on hepatic aryl hydrocarbon hydroxylase activity.

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Materials and Methods

Chemicals

(G-^{3}H) Benzo(a)pyrene with a specific activity of 23 Ci/mmole was purchased from Amersham Corporation. UICC standard reference sample of Canadian chrysotile B was obtained from the National Research Institute for Occupational Diseases, Johanessburg, South Africa. Silica (80-120 mesh) was procured from Fisher Scientific Company, Fairlawn, NJ. The sources of other chemicals have been indicated in earlier communications (*9, 10*).

Preparation of Microsomes and Enzyme Assays

Male Sprague-Dawley rats weighing between 200 and 250 g were used in these experiments. Animals were pretreated by daily IP injections of 3-methylcholanthrene (in corn oil, 40 mg/kg of body weight) for 4 days. They had free access to laboratory chow and drinking water. Microsomal fractions from liver homogenates were isolated by differential centrifugation as described earlier (*9*). Radioactive assays for aryl hydrocarbon hydroxylase were performed essentially as described by Van Cantfort et al. (*11*). (G-^{3}H) Benzo(a)pyrene (specific activity, 23 Ci/mmole) was diluted to a specific activity of 5.0 mCi/mmole by using the unlabeled material. The incubation medium for metabolic studies consisted of potassium phosphate buffer, pH 7.4 (10 *mM*), NADPH (0.4 *mM*), $MgCl_2$ (1.1 *mM*), benzo(a)pyrene (80 μM), and enzyme preparation (about 50 μg of protein) in a final volume of 2.0 mL. The period of incubation was usually 15 min at 37°C. The products were extracted twice with two volumes of chilled ethyl acetate:acetone (2:1, v/v), and the metabolites were separated and identified by thin-layer chromatography on Silica Gel G plates, following standard procedures (*12, 13*). Microsomal ethoxyresorufin deethylase activity was assayed as described by Pohl and Fouts (*14*).

Animal Experiments

Male Sprague-Dawley rats weighing between 125 and 150 g were divided into two groups of three each. One group received intraperitoneal injections of chrysotile (5.0 mg suspended in 1.0 mL of sterile saline per animal). Saline was administered intraperitoneally to the second group of rats, which served as control. The rats had free access to laboratory chow and water. The injection schedule was repeated after three days. The rats were maintained for a further 5-day period. Thereafter they were sacrificed by decapitation and their livers were excised. The individual liver tissues were subjected to subcellular fractionation and the microsomal fractions were isolated as described earlier (*9*). Aryl hydrocarbon hydroxylase was assayed in these fractions as described elsewhere.

Results and Discussion

Earlier studies (*9, 10*) illustrated that asbestos fibers impaired aryl hydrocarbon hydroxylase activity of rat liver and lung microsomes *in vitro*. Further experiments reveal that chrysotile asbestos inhibits the overall metabolism of benzo(a)pyrene *in vitro*. A perusal of Table 1 would reveal that the formation of organic-soluble benzo(a)pyrene metabolites is considerably reduced in the presence of chrysotile. Leaching chrysotile in deionized water for 24 hr causes a similar effect. The effects of silica, chrysotile, and water-leached chrysotile on the total microsomal metabolism of benzo(a)pyrene are depicted in Table 2. Chrysotile, as well as water-leached chrysotile, inhibits the total metabolism of benzo(a)pyrene. Silica, in contrast to chrysotile asbestos, does not diminish the microsomal metabolism of benzo(a)pyrene. Water leaching has been shown to release Mg^{2+} and Ca^{2+} from the asbestos fibers into the supernatant (*15*). It would appear, therefore, that the modifying effect of chrysotile on benzo(a)pyrene metabolism is not directly related to its ionic composition.

Table 1. Effect of pretreatment of microsomes with chrysotile on the pattern of benzo(a)pyrene metabolites produced.[a]

Condition	Metabolite, nmole/mg protein			
	Polar	Diols	Phenols	Quinones
Control	1.68	1.19	0.63	0.80
Chrysotile-pretreated	0.90	0.52	0.27	0.21

[a]3-Methylcholanthrene-pretreated rat liver microsomes were preincubated with chrysotile asbestos (1.0 mg/mL) for 1 hr at 37°C. Ethyl acetate-extractable metabolites are expressed at nmoles formed per mg of protein during a 15-min incubation period.

Table 2. Effect of pretreatment of microsomes with chrysotile, silica, and water-leached chrysotile on the total metabolism of benzo(a)pyrene.[a]

Treatment	Benzo(a)pyrene metabolized, (nmole/mg of protein/min[b]
None	1.11
Silica	1.25
Chrysotile	0.75
Leached chrysotile	0.71

[a]3-Methylcholanthrene-pretreated rat liver microsomes were used in the assays. Microsomes were preincubated with the particles (1.0 mg/mL) for 1 hr at 37°C. Chrysotile was leached in deionized water for 24 hr. Benzo(a)pyrene metabolites were extracted into ethyl acetate:acetone (2:1; v/v) and separated by thin-layer chromatography.

[b]The total amount of benzo(a)pyrene metabolites formed in both the organic and aqueous phases is given.

Chrysotile appears to depress the microsomal production of water-soluble benzo(a)pyrene metabolites, as depicted in Table 3. The same effect is noticed with water-leached chrysotile. However, silica does not seem to have any effect on the microsomal generation of water-soluble metabolites of benzo(a)pyrene. Precoating chrysotile with heparin or bovine serum albumin seems to partially abolish its inhibitory effect on the microsomal metabolism of benzo(a)pyrene. The coating process which makes the asbestos more hydrophobic might reduce the *in vitro* reactivity of the fibers. Interactions between the fiber surface and microsomal mixed-function oxidase are conceivable. Treatment of 3-methylcholanthrene-pretreated rat liver microsomes with chrysotile decreases the activity of microsomal ethoxyresorufin deethylase as illustrated in Table 4. Since the fibers could modify the activities of other drug-metabolizing enzymes, the alteration of microsomal metabolism of benzo(a)pyrene could not entirely be explained on the basis of simple adsorption of the hydrocarbon onto the surface of the asbestos fiber.

Table 3. Effect of pretreatment of microsomes with chrysotile and silica on the production of water-soluble benzo(a)pyrene metabolites.[a]

Treatment	Metabolite formed, nmole/mg protein[b]
None	13.51
Chrysotile	9.86
Silica	13.68

[a]3-Methylcholanthrene-pretreated microsomes were used in these assays. The microsomes were preincubated with the particles (1.0 mg/mL) for 1 hr at 37°C. Organic soluble metabolites of benzo(a)pyrene were removed by extraction of the incubation mixtures with ethyl acetate:acetone (2:1; v/v).

[b]Metabolites in the aqueous phase are expressed as nmoles formed per mg of protein during a 30-min incubation period.

Table 4. Effect of pretreatment of rat liver microsomes with chrysotile on ethoxyresorufin deethylase activity.[a]

Condition	Fluorescence, %[b]
Control	72.0 (62-76)
Chrysotile-treated	40.0 (37-45)

[a]Rat liver microsomes were pretreated with chrysotile as described in Table 1.

[b]Percentage of fluorescence intensity is given. The values represent averages of four determinations. The range is given in parentheses.

Table 5. Effect of chrysotile administration to rats intraperitoneally on liver microsomal aryl hydrocarbon hydroxylase activity.[a]

Treatment	Benzo(a)pyrene metabolized, nmole/mg protein/min[b]
Saline	0.79
Chrysotile	0.46

[a]Aryl hydrocarbon hydroxylase activity of liver microsomes was determined by a radioactive assay as described in "Materials and Methods" section.

[b]Expressed as nmole of benzo(a)pyrene metabolites formed per mg of protein per minute. The values represent averages of duplicate determinations for each group of three rats.

Treatment of rats with chrysotile seems to have an effect on the microsomal metabolism of benzo(a)pyrene. In this experiment the rats received intraperitoneal administration of chrysotile. This manipulation seems to reduce the liver microsomal aryl hydrocarbon hydroxylase activity to 58% of that in the control, as depicted in Table 5. In this experiment, the hydroxylase activity was assayed radioactively by measuring the formation of total benzo(a)pyrene metabolites. The results, therefore, indicate that asbestos could inhibit the total metabolism of benzo(a)pyrene in liver microsomes.

Subcutaneous injection of asbestos into the flanks of mice resulted in fibers in lymph nodes, spleen, liver, kidneys, and brain (*16*). Among asbestos workers, Langer (*17*) found asbestos fibers and bodies in the liver, pancreas, kidney, spleen and adrenal glands. Although the fibers were present in smaller quantities than in the lung, it is possible that extrapulmonary organs are more sensitive to the effects of asbestos than is lung tissue. In the present study, the presence of asbestos fibers in the liver has not been demonstrated. However, intraperitoneally administered asbestos is known to migrate to various organs of the rat (*18*). If the inhibition of microsomal aryl hydrocarbon hydroxylase activity, observed in the present study, were due to chrysotile, this effect would have important consequences. The interference of asbestos with the detoxification of benzo(a)pyrene may result in the increased residence time of the carcinogen. The modification of benzo(a)pyrene-metabolizing enzymes by asbestos might be an important event in its action as a cocarcinogen. It is conceivable that mechanisms other than particle-enhanced uptake of carcinogen (*19*) are operative in polynuclear aromatic hydrocarbon and asbestos cocarcinogenesis.

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